2 SYNOPSIS

BioMarin Pharmaceutical Inc. **Finished Product:** Phenoptin™ **Active Ingredient:** sapropterin dihydrochloride	Individual Study Table Referring to Part of the Dossier Volume: Page:
Protocol No. PKU-001: A Phase 2, Multicenter, Open-Label Study to Evaluate the Response to and Safety of an 8-Day Course of Phenoptin™ (sapropterin dihydrochloride) Treatment in Subjects with Phenylketonuria Who Have Elevated Phenylalanine Levels	
Principal Investigators/Study Centers: [redacted], University of British Columbia Children's Women's Hospital; [redacted] Hospital for Sick Children; [redacted] Hôpital Necker-Enfants Malades; [redacted] Hôpital Jeanne de Flandres; François Feillet, Hôpital d'Enfants; Julia Hennermann, Otto-Heubner-Centrum für Kinder-und Jungendmedizin; [redacted] Klinik für Kinder-und Jugendmedizin Reutlingen; [redacted] Universitätsklinikum der Heinrich-Heine; [redacted] The Children's University Hospital; [redacted] Azienda Ospedaliera San Paolo; [redacted] Azienda Ospedaliera Universitaria; [redacted] Instytut Matki I Dziecka; [redacted] Birmingham Children's Hospital; [redacted] Great Ormond Street Hospital; [redacted] National Hospital for Neurology and Neurosurgery; [redacted] Royal Manchester Children's Hospital; [redacted] Children's Memorial Hospital; [redacted] University of California Los Angeles; Dorothy Grange, Washington University School of Medicine; [redacted] Oregon Health & Science University; [redacted] Children's Hospital & Research Center at Oakland; [redacted] Children's Hospital of Boston; [redacted] University of Utah; [redacted] Children's Hospital Los Angeles; [redacted] Yale University School of Medicine; [redacted] University of Pittsburgh Medical Center; [redacted] University of Texas Southwestern Medical Center; [redacted] Mount Sinai School of Medicine; [redacted] University of Minnesota; [redacted] University of Wisconsin-Madison	
Publication:	

Study Duration:	36 Days	**Development Phase:** 2
Report Period:	48 weeks	
First Enrollment:	04 December 2004 (first consent)	
Last Dose Given:	07 October 2005	

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Objectives: The primary objective of this study was as follows:

- To evaluate the degree and frequency of response to Phenoptin (sapropterin dihydrochloride) treatment, as demonstrated by a reduction in blood phenylalanine (Phe) level, among subjects with PKU who have elevated blood Phe levels

The secondary objectives of this study were as follows:

- To identify individuals in this subject population who respond to Phenoptin treatment with a reduction in blood Phe level at Day 8
- To evaluate the safety of Phenoptin treatment in this subject population

The responders in Study PKU-001 were eligible to enroll in the pivotal Study PKU-003. Study PKU-003 is a randomized, placebo-controlled Phase 3 trial designed to demonstrate efficacy of Phenoptin in reducing blood Phe levels after 6 weeks of treatment.

Methodology: This Phase 2, multicenter, open-label study was designed to evaluate the degree and frequency of response to Phenoptin and the safety of Phenoptin in subjects with PKU who have elevated blood Phe levels (≥450 µmol/L) and to identify 80-100 subjects with a ≥ 30% reduction in blood Phe level at Day 8 who would be eligible for additional studies with Phenoptin. Within 6 weeks of completing screening assessments to determine eligibility, subjects began treatment with 10 mg/kg Phenoptin, administered orally once daily for 8 days (Days 1–8). Subjects were instructed to continue their usual diet without modification (ie, no increase or decrease in Phe restriction). Blood Phe levels were measured on the following days: Screening, Day 1 (just prior to the first dose), and Day 8. A central laboratory determined blood Phe levels. Safety was assessed by medical history and physical examinations, adverse events and vital signs, and clinical laboratory tests (chemistry, hematology, thyroid function tests, and urinalysis). In addition, blood samples were collected at screening for *PAH* gene mutation analysis.

Number of Patients:	Planned: up to 700; Actual number enrolled = 490

Diagnosis and Main Criteria for Inclusion: Individuals eligible to participate in this study included those who met all of the following criteria:

- Age ≥ 8 years
- Blood Phe level at screening of ≥ 600 µmol/L (original protocol) or ≥ 450 µmol/L (after approval of Amendment 2)

Individuals were not eligible to participate in this study if they met any of the following criteria:

- ALT >5 times the upper limit of normal (ie, Grade 3 or higher based on World Health Organization Toxicity Criteria) at Screening
- Clinical diagnosis of primary BH4 deficiency

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Test Product, Dose and Mode of Administration, Batch Number: Phenoptin was supplied as white, off-white, or light yellow 300 mg tablets, each containing 100 mg of sapropterin dihydrochloride. Phenoptin was packaged in blister cards containing 28 tablets per card. Phenoptin was administered orally once daily at a dose of 10 mg/kg, dissolved in 4–8 oz (120 to 240 mL) of water, orange juice, or apple juice. The first dose was taken at the study site; all other doses were to be taken approximately 5–10 minutes before the morning meal. Batch number [redacted] was used in the U.S. and Canada; batch numbers [redacted] & [redacted] were used in Europe.

Duration of Treatment: Subjects were to receive Phenoptin for 8 consecutive days.

Reference Therapy, Dose and Mode of Administration, Batch Number: None

Criteria for Evaluation:

Primary Outcome Variable: The primary outcome variable was response to Phenoptin on Day 8, defined as a reduction in blood Phe level of ≥ 30% compared with the Day 1 measurement.

Safety: Safety was evaluated on the basis of incidence of adverse events and clinically significant changes in laboratory test results (chemistry, hematology, thyroid function tests, and urinalysis).

Pharmacokinetics: Not measured as part of this study

Statistical Methods:

Primary Outcome Variable: The primary outcome variable was response to Phenoptin on Day 8, with response defined as a reduction in blood Phe level of ≥ 30% compared with baseline (Day 1). A subject's percentage change in blood Phe level at Day 8 was calculated as:

[(Day 8 blood Phe level – Day 1 blood Phe level) / Day 1 blood Phe level] x 100%.

A subject who had a reduction in blood Phe level of at least 30% was defined as a Responder.

The proportion of Responders (p) was calculated as the number of Responders divided by the number of subjects enrolled (n) who had both Day 1 and Day 8 blood Phe level measurements. The standard error (se) was calculated as $\sqrt{p(1-p)/n}$. The 95% confidence limits were calculated using the normal approximation by p ± 1.96 x se.

The original sample size was based on the desired precision of the estimated response rates. If the observed response rate was 30%, the 95% confidence interval (CI) was expected to be 26%-35%. Ultimately, only 490 subjects were needed to identify 96 subjects who had a ≥ 30% reduction in blood Phe at Day 8.

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Summary of Results:

Patient Population: A total of 490 subjects were enrolled at 30 sites, 16 in North America and 14 in Europe. Each site enrolled between 4 and 35 subjects. Males comprised 48% of the population. The mean (± SD) age was 21.8 ± 8.9 years (range 8 to 48 years); mean standing height was 164.0 ± 12.3 cm (range 120 to 194 cm); and mean weight was 69.5 ± 22.3 kg (range 21 to 166 kg). A large proportion (96%) of the subjects were Caucasian.

Primary Efficacy Endpoints: Of the 485 subjects with blood Phe measurements at Day 1 and Day 8, 96 (20%, 95% CI of [16%, 23%]) were Responders. These Responders had a mean change of −391.8 ± 185.3 µmol/L in blood Phe between Day 1 and Day 8. The mean percent change in Responders was −50.0 ± 16.0. The mean percent changes were similar between the group with baseline blood Phe levels < 600 µmol/L and the group with ≥ 600 µmol/L (−55.3 ± 16.7 and −47.5 ± 15.2, respectively). Blood Phe levels decreased overall, with a mean percent change of −11.4 ± 25.5. Subjects with baseline blood Phe levels < 600 µmol/L had a greater mean percent reduction than subjects with higher (≥ 600 µmol/L) baseline blood Phe levels (−25.4 ± 39.3% vs −9.6 ± 22.5%, respectively).

Extent of Exposure: Subjects received a mean daily dose of 743.9 ± 224 mg. Subjects with a baseline blood Phe level < 600 µmol/L had a slightly lower mean daily dose than those in the ≥ 600 µmol/L group, reflecting the mean lower weight in this group. The mean daily dose in mg/kg was identical in the 2 groups.

Safety: Of the 489 subjects who received at least 1 dose of Phenoptin, 233 (48%) experienced an AE between Day 1 and Day 36. No deaths occurred in the study. One AE occurred (pregnancy) that required the subject to withdraw from the study.

Of the 233 subjects who experienced an AE at any time after the first dose of Phenoptin, 157 (67%) experienced an AE that was judged by the PI to be possibly or probably related to Phenoptin. The frequency of Phenoptin-related AEs was 58% (281 of 482 AEs). Most AEs were rated as mild in intensity; only 4 subjects had AEs that were considered severe. In general, subjects with higher baseline (Day 1) blood Phe levels were more likely to experience an AE, experience a severe AE, and have an AE judged as Phenoptin related. One AE, appendicitis, was considered to be an SAE. This event occurred 2 weeks after the last dose of Phenoptin and was judged by the investigator to be unrelated to Phenoptin administration.

No overall trends were observed in serum chemistry and hematology laboratory results. The majority of laboratory results that were considered clinically significant occurred at Screening or on Day 1, prior to administration of the first dose of Phenoptin. The most common clinically significant treatment-emergent laboratory results were increased levels of liver enzymes, cholesterol, uric acid, and bilirubin, and decreases in white blood cells and platelets.

Conclusions: In this study, 20% of individuals with PKU responded to an 8-day course of a once daily 10 mg/kg dose of Phenoptin with a reduction in blood Phe of at least 30%. The safety data indicated an acceptable risk-benefit profile, with adverse events that were generally mild and minimal treatment-emergent laboratory abnormalities occurring during the study.

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Status of Study: Completed	
Date of Study Report:	22 December 2006